10/509065
RO/US 22 APR 2003
Rec'd PCT/PTO 24 SEP 2004
PCT/US03/07443.22042003

USE OF CARBON-2-MODIFIED-19-NOR-VITAMIN D ANALOGS TO INDUCE THE FORMATION OF NEW BONE

BACKGROUND OF THE INVENTION

The present invention relates to vitamin D compounds, and more particularly to 19-nor vitamin D compounds substituted at the carbon 2 position which are useful for stimulating growth of new bone.

The ability of vitamin D to bring about normal bone formation is well recognized and has been for well over 75 years. Thus, vitamin D will heal rickets and osteomalacia. In the case of these two diseases, it is envisioned that the osteoblasts of bone are able to synthesize the organic matrix of the skeleton even in the absence of vitamin D but that vitamin D is required for the deposit of mineral in the newly-layed down matrix. In this capacity, it is generally believed that vitamin D heals rickets and osteomalacia by the elevation of plasma calcium and phosphorus to levels required for the mineralization process to proceed (DeLuca[1], 1981). Thus, early work (Shipley, Kramer, and Howland,[2,3] 1925; 1926) suggested that serum taken from normal rats could heal rachitic lesions in culture, whereas serum taken from rachitic rats was unable to bring about the same healing process. Later, it was discovered that this was because vitamin D by virtue of its ability to elevate the absorption of calcium and phosphorus in the small intestine, is able to raise the plasma calcium and phosphorus to supersaturation levels required for the mineralization of the skeleton. Furthermore, it was envisioned that vitamin D also could cause the mobilization of calcium from bone to elevate plasma calcium concentration (DeLuca[1], 1981) or could stimulate the kidney to reabsorb calcium from the formed urine (Yamamoto et al.[4], 1984) raising the plasma calcium and phosphorus product needed for the mineralization process. Final proof that this is the case was provided when calcium and phosphorus infusion into the blood stream

of vitamin D-deficient rats brought about normal mineralization of organic matrix of bone and that vitamin D contributed little beyond that if any (Underwood and DeLuca[5], 1984).

In the intervening time between the work of Shipley, Kramer, and Howland,[2,3] (1925; 1925) and the work carried out by Underwood and DeLuca[5] (1984), a great deal of information was derived regarding how vitamin D carries out its functions. It is now abundantly clear that vitamin D must first be 25-hydroxylated in the liver and subsequently 1α-hydroxylated in the kidney before it can function (DeLuca[6], 1974). These two reactions produce the final active form of vitamin D, namely 1,25-$(OH)_2D_3$ (DeLuca & Schnoes[7], 1983). This compound then stimulates the intestine to absorb calcium, the kidney to reabsorb calcium, the intestine to absorb phosphate, and it stimulates bone to mobilize calcium when signaled by high parathyroid hormone levels. These actions result in a rise in plasma calcium and phosphorus levels that bring about the healing of bone lesions such as rickets and osteomalacia and prevent the neurological disorder of hypocalcemic tetany.

1,25-$(OH)_2D_3$, therefore, has been used in a variety of bone diseases; among them are the treatment of renal osteodystrophy, osteoporosis, osteomalacia, and various types of rickets (Feldman D, Glorieux FH, Pike JW, eds.[8], 1997). In addition, it has been used to treat hypocalcemia of hypoparathyroid patients (Kooh et al.[9], 1975). To treat secondary hyperparathyroidism of renal osteodystrophy, it is well known that this hormone binds to the vitamin D receptor (VDR) located in the parathyroid glands to suppress both growth and proliferation of the parathyroid cells and expression of the preproparathyroid gene (Demay et al.[10], 1992; Darwish & DeLuca[11], 1999) . However, the use of 1,25-$(OH)_2D_3$ to promote new bone growth has never been envisioned and, in fact, treatment of post-menopausal women with 1,25-$(OH)_2D_3$ will decrease the fracture rate but will not appreciably

increase the bone mass (Aloia[12], 1990; Tilyard et al.[13], 1992). Therefore, an anabolic action of $1,25\text{-}(OH)_2D_3$ on bone is unknown and, in fact, evidence is to the contrary.

Bone turnover is a normal critical process that is homeostatic in nature and necessary for renewal of defective bone that occurs as a result of normal aging or trauma. This process is essential to the maintenance of adult skeletal integrity and is carried out through the activity of two important cell types, the bone resorbing osteoclast and the bone forming osteoblast. Steroid hormones such as vitamin D play an important modulatory role in the regulation of osteoblast production and function.

Currently, the treatment of bone loss disorders utilizes anti-bone resorption substances. The estrogens for example are used to treat post-menopausal osteoporosis through their capacity to block bone resorption that results from the lack of female hormones. The bis-phosphonates which include Fosamax® act by blocking the resorption of bone, thus causing an increase in bone mass. It is very clear, therefore, that the anti-resorption agents cannot be considered for use under circumstances where new bone growth is required.

During the course of investigating analogs of $1,25\text{-}(OH)_2D_3$, it was discovered that modifying the vitamin D hormone on the 2-carbon could have very profound effects on its biological activity (Sicinski et al.[14], 1998). For example, in U.S. Pat. No. 4,666,634, 2β-hydroxy and alkoxy (e.g., ED-71) analogs of 1α,25-dihydroxyvitamin D_3 have been described and examined as potential drugs for osteoporosis and as antitumor agents. See also Okano et al., Biochem. Biophys. Res. Commun. 163, 1444 (1989). Other 2-substituted (with hydroxyalkyl, e.g., ED-71, and fluoroalkyl groups) A-ring analogs of 1α,25-dihydroxyvitamin D_3 have also been prepared and tested (Miyamoto et al., Chem. Pharm. Bull. 41, 1111 (1993); Nishii et al., Osteoporosis Int. Suppl. 1, 190 (1993); Posner et al., J. Org.

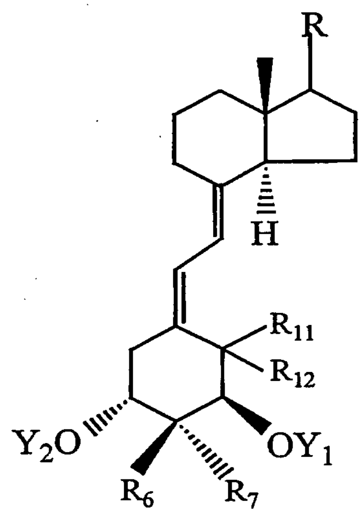
Chem. 59, 7855 (1994), and J. Org. Chem. 60, 4617 (1995)). This was especially true in the 19-nor analogs where these compounds have been found to increase bone strength and increase bone mass in ovariectomized rats (see DeLuca U.S. Patent No. 6,306,844), and is especially true of 2α-methyl-19-nor-20S-1,25-$(OH)_2D_3$ and 2-methylene-19-nor-20S-1,25-$(OH)_2D_3$. It is not clear how these compounds increase bone mass or improve bone strength. It is, indeed, possible that they act as anti-resorptive substances because they could diminish the parathyroid hormone levels by suppression of the parathyroid glands which would in turn diminish bone resorption. Other 2-substituted analogs of 1α,25-dihydroxy-19-norvitamin D_3 have also been synthesized, i.e. compounds substituted at 2-position with hydroxy or alkoxy groups (DeLuca et al., U.S. Pat. No. 5,536,713), which exhibit interesting and selective activity profiles. Further, the teachings in the literature argue that vitamin D compounds are not required and do not increase the synthesis of new bone.

SUMMARY OF THE INVENTION

It has now been found that 2-carbon-modified vitamin D compounds can markedly stimulate the formation of new bone when added to primary cultures of osteoblasts and its precursors. This activity is selective since the native hormone, 1,25-$(OH)_2D_3$, does not produce this effect. These results demonstrate that this feature is a unique property of the 2-carbon-modified analogs of 1,25-$(OH)_2D_3$, and more specifically these compounds can be used to stimulate new bone growth and be used to stimulate osteoblastic-mediated bone growth. As a result, these compounds can be used to markedly increase the rate of skeletal repairs such as repair of fractures, osseointegration of transplants, and the solidification of implants as well as acceleration of and improvement of bone quality following distraction osteogenesis procedures. These compounds will also find use in improving

surgical outcomes employing a variety of orthopedic devices and dental implants. The present invention is thus directed toward various pharmaceutical uses for 2-carbon modified analogs of vitamin D compounds which involve the formation of new bone.

Structurally these 2-carbon modified analogs of vitamin D compounds are characterized by the general formula I shown below:

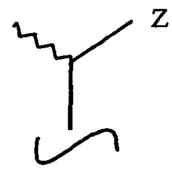


I

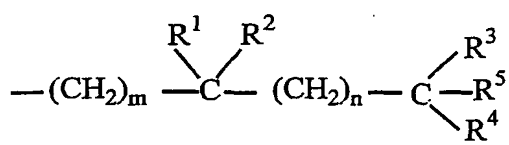
where Y_1 and Y_2, which may be the same or different, are each selected from the group consisting of hydrogen and a hydroxy-protecting group, where R_{11} and R_{12} are each hydrogen or taken together are a methylene group, where R_6 and R_7,. which may be the same or different, are each selected from the group consisting of hydrogen, alkyl, hydroxyalkyl, fluoroalkyl, hydroxy and alkoxy, with the proviso that R_6 and R_7 cannot both be hydrogen, or R_6 and R_7 when taken together may represent the group $-(CH_2)_x-$ where X is an integer from 2 to 5, or R_6 and R_7 when taken together may represent the group $=CR_8R_9$ where R_8 and R_9, which may be the same or different, are each selected from the group consisting of hydrogen, alkyl, hydroxyalkyl, fluoroalkyl, hydroxy and alkoxy, or when taken together R_8 and R_9 may represent the group $-(CH_2)_x-$ where X is an integer from 2 to 5, and where the

group R represents any of the typical side chains known for vitamin D type compounds.

More specifically R can represent a saturated or unsaturated hydrocarbon radical of 1 to 35 carbons, that may be straight-chain, branched or cyclic and that may contain one or more additional substituents, such as hydroxy- or protected-hydroxy groups, fluoro, carbonyl, ester, epoxy, amino or other heteroatomic groups. Preferred side chains of this type are represented by the structure below



where the stereochemical center (corresponding to C-20 in steroid numbering) may have the R or S configuration, (i.e. either the natural configuration about carbon 20 or the 20-epi configuration), and where Z is selected from Y, -OY, $-CH_2OY$, -C≡CY and -CH=CHY, where the double bond may have the cis or trans geometry, and where Y is selected from hydrogen, methyl, $-COR^5$ and a radical of the structure:

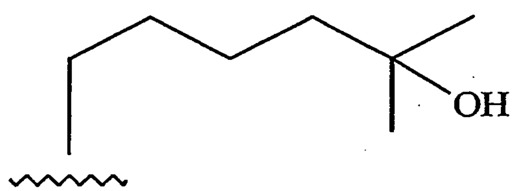


where m and n, independently, represent the integers from 0 to 5, where R^1 is selected from hydrogen, deuterium, hydroxy, protected hydroxy, fluoro, trifluoromethyl, and C_{1-5}-alkyl, which may be straight chain or branched and, optionally, bear a hydroxy or protected-hydroxy substituent, and where each of R^2, R^3, and R^4, independently, is selected from deuterium, deuteroalkyl, hydrogen, fluoro, trifluoromethyl and C_{1-5} alkyl, which may be straight-chain or branched, and optionally, bear a hydroxy or protected-hydroxy substituent, and where R^1 and R^2, taken together, represent an oxo

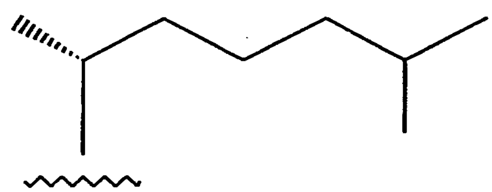
group, or an alkylidene group, $=CR^2R^3$, or the group $-(CH_2)_p-$, where p is an integer from 2 to 5, and where R^3 and R^4, taken together, represent an oxo group, or the group $-(CH_2)_q-$, where q is an integer from 2 to 5, and where R^5 represents hydrogen, hydroxy, protected hydroxy, or C_{1-5} alkyl and wherein any of the CH-groups at positions 20, 22, or 23 in the side chain may be replaced by a nitrogen atom, or where any of the groups $-CH(CH_3)-$, $-(CH_2)_m-$, $-CR_1R_2-$ or $-(CH_2)_n-$ at positions 20, 22, and 23, respectively, may be replaced by an oxygen or sulfur atom.

The wavy line to the methyl substituent at C-20 indicates that carbon 20 may have either the R or S configuration.

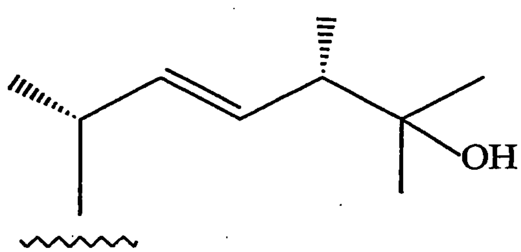
Specific important examples of side chains with natural 20*R*-configuration are the structures represented by formulas **(a)**, **b)**, **(c)**, **(d)** and **(e)** below. i.e. the side chain as it occurs in 25-hydroxyvitamin D_3 **(a)**; vitamin D_3 **(b)**; 25-hydroxyvitamin D_2 **(c)**; vitamin D_2 **(d)**; and the C-24 epimer of 25-hydroxyvitamin D_2 **(e)**:



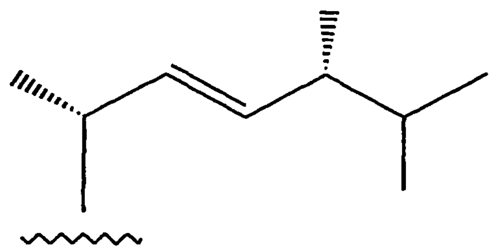
(a)



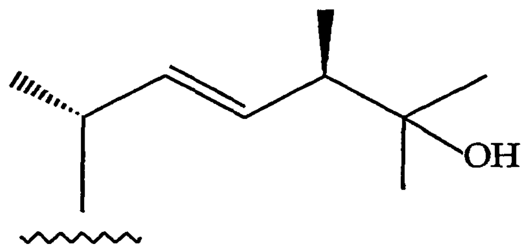
(b)



(c)



(d)



(e)

Preferred compounds are the 2-carbon modified analogs of 19-nor-1α,25-dihydroxyvitamin D_3, particularly 2-methylene-19-nor-20(S)-1α,25-dihydroxyvitamin D_3 and 2α-methyl-19-nor-20(S)-1α,25-dihydroxyvitamin D_3. Slow release forms of these compounds are also desirable, i.e. compounds having an acyl group at positions 1, 3 and/or 25, particularly 25-acetate forms.

The above compounds exhibit a desired, and highly advantageous, pattern of biological activity. These compounds are characterized by their ability to stimulate new bone growth and thus may be used to stimulate osteoblastic-mediated bone growth. Their activity on stimulating new bone growth allows the in vivo administration of these compounds as preferred therapeutic agents for the healing of bone fractures, for the healing of bone transplants, for the solidification of implants in bone, for the osseointegration of dental implants, and to stimulate growth of periodontal bone. The treatment may be topical, transdermal, oral or parenteral. The compounds may be present in a composition in an amount from about 0.01μg/gm to about 50μg/gm of the composition, and may be administered in dosages of from about 0.01μg/day to about 50μg/day.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1a, 1b and 1c are photographs of osteoblast cultures after 14 days of incubation showing the effect on osteoblasts of control (Fig. 1a), and a 10^{-8} molar concentration of 1α,25-dihydroxyvitamin D_3 (Fig. 1b), and a 10^{-8} molar concentration of 2-methylene-19-nor-20(S)-1α,25-hydroxyvitamin D_3 (Fig. 1c); and

Figures 2a, 2b, 2c, 2d and 2e are photographs of Von Kossa stained osteoblast cultures showing calcified bone in the form of dark nodules as a result of treatment with control (Fig. 2a), a 10^{-8} molar concentration of 1α,25-dihydroxyvitamin D_3 (Fig. 2b), a 10^{-10} molar concentration of 1α,25-dihydroxyvitamin D_3 (Fig. 2c), a 10^{-10} molar concentration of 2-methylene-19-nor-20(S)-1α,25-dihydroxyvitamin D_3 (Fig. 2d), and a 10^{-12} molar concentration of 2-methylene-19-nor-20(S)-1α,25-dihydroxyvitamin D_3 (Fig. 2e).

DETAILED DESCRIPTION OF THE INVENTION

As used in the description and in the claims, the term "hydroxy-protecting group' signifies any group commonly used for the temporary protection of hydroxy functions, such as for example, alkoxycarbonyl, acyl, alkylsilyl or alkylarylsilyl groups (hereinafter referred to simply as "silyl" groups), and alkoxyalkyl groups. Alkoxycarbonyl protecting groups are alkyl-O-CO- groupings such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, isopropoxycarbonyl, butoxycarbonyl, isobutoxycarbonyl, tert-butoxycarbonyl, benzyloxycarbonyl or allyloxycarbonyl. The term "acyl" signifies an alkanoyl group of 1 to 6 carbons, in all of its isomeric forms, or a carboxyalkanoyl group of 1 to 6 carbons, such as an oxalyl, malonyl, succinyl, glutaryl group, or an aromatic acyl group such as benzoyl, or a halo, nitro or alkyl substituted benzoyl group. The word "alkyl" as used in the description or the claims, denotes a straight-chain or branched alkyl radical of 1 to 10 carbons, in all

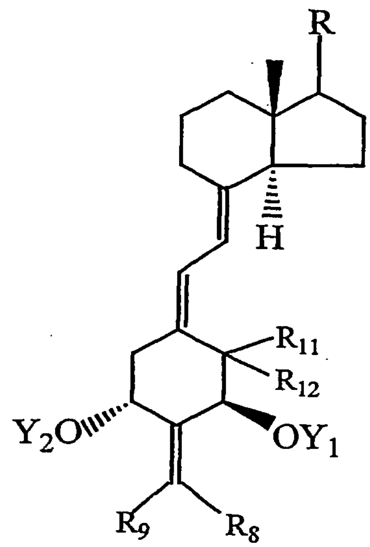
its isomeric forms. Alkoxyalkyl protecting groups are groupings such as methoxymethyl, ethoxymethyl, methoxyethoxymethyl, or tetrahydrofuranyl and tetrahydropyranyl. Preferred silyl-protecting groups are trimethylsilyl, triethylsilyl, t-butyldimethylsilyl, dibutylmethylsilyl, diphenylmethylsilyl, phenyldimethylsilyl, diphenyl-t-butylsilyl and analogous alkylated silyl radicals. The term "aryl" specifies a phenyl-, or an alkyl-, nitro- or halo-substituted phenyl group, and the term "alkoxy" specifies an -O-alkyl group.

A "protected hydroxy" group is a hydroxy group derivatised or protected by any of the above groups commonly used for the temporary or permanent protection of hydroxy functions, e.g. the silyl, alkoxyalkyl, acyl or alkoxycarbonyl groups, as previously defined. The terms "hydroxyalkyl", "deuteroalkyl" and "fluoroalkyl" refer to an alkyl radical substituted by one or more hydroxy, deuterium or fluoro groups respectively.

It should be noted in this description that the term "24-homo" refers to the addition of one methylene group and the term "24-dihomo" refers to the addition of two methylene groups at the carbon 24 position in the side chain. Likewise, the term "trihomo" refers to the addition of three methylene groups. Also, the term "26,27-dimethyl" refers to the addition of a methyl group at the carbon 26 and 27 positions so that for example R^3 and R^4 are ethyl groups. Likewise, the term "26,27-diethyl" refers to the addition of an ethyl group at the 26 and 27 positions so that R^3 and R^4 are propyl groups. When R_{11} and R_{12} are both hydrogen, the compounds are referred to herein as "19-nor" compounds.

2-Alkylidene Compounds

Structurally these 2-alkylidene analogs are characterized by the general formula V shown below:



V

where Y_1 and Y_2, which may be the same or different, are each selected from the group consisting of hydrogen and a hydroxy-protecting group, R_{11} and R_{12} are both hydrogen or taken together are a methylene group, R_8 and R_9, which may be the same or different, are each selected from the group consisting of hydrogen, alkyl, hydroxyalkyl and fluoroalkyl, or, when taken together represent the group $-(CH_2)_X-$ where X is an integer from 2 to 5, and where the group R represents any of the typical side chains known for vitamin D type compounds as previously described herein.

In the following lists of compounds, the particular alkylidene substituent attached at the carbon 2 position should be added to the nomenclature. For example, if a methylene group is the alkylidene substituent, the term "2-methylene" should preceed each of the named compounds. If an ethylene group is the alkylidene substituent, the term "2-ethylene" should preceed each of the named compounds, and so on. In addition, if the methyl group attached at the carbon 20 position is in its epi or unnatural configuration, the term "20(S)" or "20-epi" should be included in each of the following named compounds. Also, if the side chain contains an oxygen atom substituted at any of positions 20, 22 or 23, the term “20-oxa,” “22-oxa” or “23-oxa,” respectively,

should be added to the named compound. The named compounds could also be of the vitamin D_2 type if desired.

Specific and preferred examples of the 2-alkylidene-compounds of structure V when the side chain is unsaturated and R_{11} and R_{12} are both hydrogen are:

19-nor-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-24-homo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-24-dihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-24-trihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-dimethyl-24-homo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-dimethyl-24-dihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-dimethyl-24-trihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-diethyl-24-homo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-diethyl-24-dihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-diethyl-24-trihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-dipropoyl-24-homo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-dipropyl-24-dihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

and

19-nor-26,27-dipropyl-24-trihomo-1,25-dihydroxy-22-dehydrovitamin D_3.

Specific and preferred examples of the 2-alkylidene-compounds of structure V when the side chain is saturated and R_{11} and R_{12} both hydrogen are:

19-nor-1,25-dihydroxyvitamin D_3;

19-nor-24-homo-1,25-dihydroxyvitamin D_3;

19-nor-24-dihomo-1,25-dihydroxyvitamin D_3;

19-nor-24-trihomo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-dimethyl-24-homo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-dimethyl-24-dihomo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-dimethyl-24-trihomo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-diethyl-24-homo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-diethyl-24-dihomo-1,25-dihydroxyvitamin D_3;

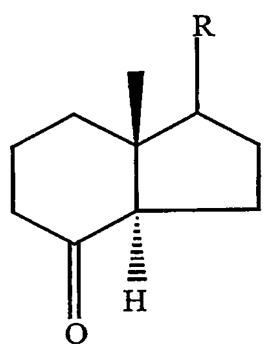
19-nor-26,27-diethyl-24-trihomo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-dipropyl-24-homo-1,25-dihydroxyvitamin D_3;

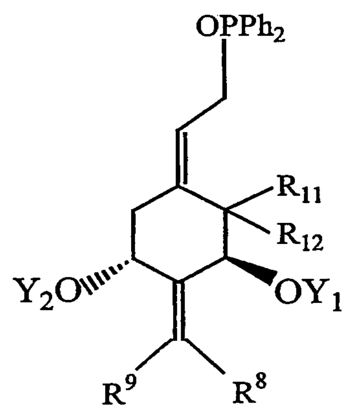
19-nor-26,27-dipropyl-24-dihomo-1,25-dihydroxyvitamin D_3; and

19-nor-26,27-dipropyl-24-trihomo-1,25-dihydroxyvitamin D_3.

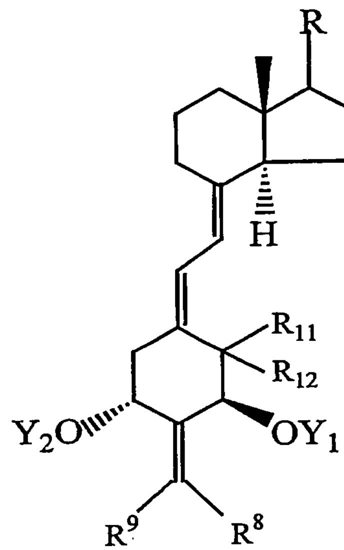
The preparation of 2-alkylidene-vitamin D compounds, particularly 2-methylene-19-nor-vitamin D compounds, having the basic structure **V** can be accomplished by a common general method, i.e. the condensation of a bicyclic Windaus-Grundmann type ketone **II** with the allylic phosphine oxide **III** to the corresponding 2-alkylidene -vitamin D analogs **IV** followed by deprotection at C-1 and C-3 in the latter compounds:



II



III



IV

In the structures **II**, **III**, and **IV** groups Y_1, Y_2, R_{11}, R_{12} and R represent the groups defined above with respect to formula **I**; Y_1 and Y_2 are preferably hydroxy-protecting groups, it being also understood that any functionalities in R that might be sensitive, or that interfere with the condensation reaction, be suitably protected as is well-known in the art. The process shown above represents an application of the convergent synthesis concept, which has been applied effectively for the preparation of vitamin D compounds [e.g. Lythgoe et al., J. Chem. Soc. Perkin Trans. I, 590 (1978); Lythgoe, Chem. Soc. Rev. 9, 449 (1983); Toh et al., J. Org. Chem. 48, 1414 (1983); Baggiolini et al., J. Org. Chem. 51, 3098 (1986); Sardina et al., J. Org. Chem. 51, 1264 (1986); J. Org. Chem. 51, 1269 (1986); DeLuca et al., U.S. Pat. No. 5,086,191; DeLuca et al., U.S. Pat. No. 5,536,713; DeLuca et al U.S. Patent No. 5,843,928 and DeLuca et al U.S. Patent No. 5,936,133.

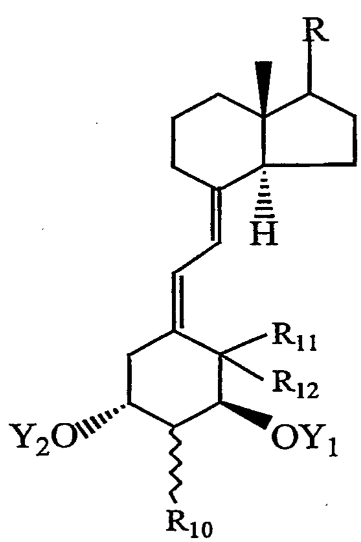
Hydrindanones of the general structure **II** are known, or can be prepared by known methods.

Also the preparation of the required phosphine oxides of general structure **III** has been developed starting from a methyl quinicate derivative, easily obtained from commercial (1R,3R,4S,5R)-(-)-quinic acid as described by Perlman et al., Tetrahedron Lett. 32, 7663 (1991) and DeLuca et al., U.S. Pat. No. 5,086,191.

C-20 epimerization may be accomplished by the analogous coupling of the phosphine oxide **III** with a (20*S*) Grundmann's ketone which after hydrolysis of the hydroxy-protecting groups will give a (20*S*)-2-alkylidene-vitamin D compound. As noted above, other 2-alkylidene-vitamin D analogs may be synthesized by the method disclosed herein, specifically for example, 2-methylene-19-nor-20(S)-1α,25-dihydroxyvitamin D_3 can be obtained wherein R_{11} and R_{12} would both be hydrogen.

2-Alkyl Compounds

Structurally these 2-alkyl analogs are characterized by the general formula **VI** shown below:



VI

where Y_1 and Y_2, which may be the same or different, are each selected from the group consisting of hydrogen and a hydroxy-protecting group, R_{11} and R_{12} are both hydrogen or taken together are a methylene group, R_{10} is selected from the group consisting of

alkyl, hydroxyalkyl and fluoroalkyl, and where the group R represents any of the typical side chains known for vitamin D type compounds as previously described herein.

In the following lists of compounds, the particular substituent attached at the carbon 2 position should be added to the nomenclature. For example, if a methyl group is the alkyl substituent, the term "2-methyl" should preceed each of the named compounds. If an ethyl group is the alkyl substituent, the term "2-ethyl" should preceed each of the named compounds, and so on. In addition, if the methyl group attached at the carbon 20 position is in its epi or unnatural configuration, the term "20(S)" or "20-epi" should be included in each of the following named compounds. Also, if the side chain contains an oxygen atom substituted at any of positions 20, 22 or 23, the term "20-oxa," "22-oxa" or "23-oxa," respectively, should be added to the named compound. The named compounds could also be of the vitamin D_2 type if desired.

Specific and preferred examples of the 2-alkyl-compounds of structure **VI** when the side chain is unsaturated and R_{11} and R_{12} are both hydrogen are:

19-nor-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-24-homo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-24-dihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-24-trihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-dimethyl-24-homo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-dimethyl-24-dihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-dimethyl-24-trihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-diethyl-24-homo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-diethyl-24-dihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-diethyl-24-trihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-dipropoyl-24-homo-1,25-dihydroxy-22-dehydrovitamin D_3;

19-nor-26,27-dipropyl-24-dihomo-1,25-dihydroxy-22-dehydrovitamin D_3;

and

19-nor-26,27-dipropyl-24-trihomo-1,25-dihydroxy-22-dehydrovitamin D_3.

With respect to the above unsaturated compounds (both 2-alkylidene and 2-alkyl compounds), it should be noted that the double bond located between the 23 and 23 carbon atoms in the side chain may be in either the (E) or (Z) configuration. Accordingly, depending upon the configuration, the term "22,23(E)" or "22,23(Z)" should be included in each of the above named compounds. Also, it is common to designate the double bond located between the 22 and 23 carbon atoms with the designation "Δ^{22}". Thus, for example, the second named compound above could also be written as 19-nor-24-homo-22,23(E)-Δ^{22}-1,25-$(OH)_2D_3$ where the double bond is the (E) configuration. Similarly, if the methyl group attached at carbon 20 is in the unnatural configuration, this compound could be written as 19-nor-20(S)-24-homo-22,23(E)-Δ^{22}-1,25-$(OH)_2D_3$.

Specific and preferred examples of the 2-alkyl-compounds of structure **VI** when the side chain is saturated and R_{11} and R_{12} are both hydrogen are:

19-nor-1,25-dihydroxyvitamin D_3;

19-nor-24-homo-1,25-dihydroxyvitamin D_3;

19-nor-24-dihomo-1,25-dihydroxyvitamin D_3;

19-nor-24-trihomo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-dimethyl-24-homo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-dimethyl-24-dihomo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-dimethyl-24-trihomo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-diethyl-24-homo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-diethyl-24-dihomo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-diethyl-24-trihomo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-dipropyl-24-homo-1,25-dihydroxyvitamin D_3;

19-nor-26,27-dipropyl-24-dihomo-1,25-dihydroxyvitamin D_3; and

19-nor-26,27-dipropyl-24-trihomo-1,25-dihydroxyvitamin D_3.

The preparation of 2-alkyl-vitamin D compounds, particularly 2α-methyl-vitamin D compounds, having the basic structure **VI** can be accomplished by a common general method, i.e. the condensation of a bicyclic Windaus-Grundmann type ketone **II** with the allylic phosphine oxide **III** to the corresponding 2-alkylidene-vitamin D analogs **IV** followed by a selective reduction of the exomethylene group at C-2 in the latter compounds to provide 2-alkyl compounds.

The process (shown above) represents an application of the convergent synthesis concept, which has been applied effectively for the preparation of vitamin D compounds. In addition to the previous references cited herein, see also DeLuca et al, U.S. Patent No. 5,945,410; DeLuca et al U.S. Patent No. 6,127,559; and DeLuca et al U.S. Patent No. 6,277,837.

The final step of the process is the selective homogeneous catalytic hydrogenation of the exomethylene unit at carbon 2 in the vitamin **IV** performed efficiently in the presence of tris(triphenylphosphine)rhodium(I) chloride [Wilkinson's catalyst, $(Ph_3P)_3RhCl$]. Such reduction conditions reduce only the C(2) methylene unit leaving C(5)-C(8) butadiene moiety unaffected. The isolated material is an epimeric mixture (ca. 1:1) of 2-alkyl-19-nor-vitamins differing in configuration at C-2. The mixture can be used without separation or, if desired, the individual 2α- and 2β-isomers can be separated by an efficient HPLC system.

The C-20 epimerization may be accomplished by the analogous coupling of the phosphine oxide **III** with a (20S) Grundmann's ketone which after hydrolysis of the hydroxy-protecting groups will give a (20S)-2-alkyl-vitamin compound.

As noted above, other 2-alkyl-vitamin D analogs may be synthesized by the method disclosed herein, specifically for example, 2α-methyl-19-nor-20(S)-1α,25-dihydroxyvitamin D_3 wherein R_{11} and R_{12} would both be hydrogen.

A number of oxa-analogs of vitamin D_3 and their synthesis are also known. For example, 20-oxa analogs are described in N. Kubodera et al, Chem. Pharm. Bull., 34, 2286 (1986), and Abe et al, FEBS Lett. 222, 53, 1987. Several 22-oxa analogs are described in E. Murayama et al, Chem. Pharm. Bull., 34, 4410 (1986), Abe et al, FEBS Lett., 226, 58 (1987), PCT International Application No. WO 90/09991 and European Patent Application, publication number 184 112, and a 23-oxa analog is described in European Patent Application, publication number 78704, as well as U.S. Patent 4,772,433.

2-Substituted Slow Release Compounds

Modified vitamin D compounds that exhibit a desirable and highly advantageous pattern of biological activity in vivo, namely, the more gradual onset and more prolonged duration of activity, may also be used herein.

Structurally, the key feature of the modified vitamin D compounds having these desirable biological attributes is that they are derivatives of 2-substituted-vitamin D analogs, in which a hydrolyzable group is attached to the hydroxy group at carbon 25 and, optionally, to any other of the hydroxy groups present in the molecule. Depending on various structural factors -- e.g. the type, size, structural complexity -- of the attached group, these derivatives hydrolyze to the active 2-substituted-vitamin D analog, at different rates in vivo, thus providing for the "slow release" of the biologically active vitamin D compound in the body.

The "slow release" in vivo activity profiles of such compounds can, of course, be further modulated by the use of mixtures of derivatives or the use of mixtures consisting of one or more vitamin D derivative together with underivatized vitamin D compounds.

It is important to stress that the critical structural feature of the vitamin derivatives identified above is the presence of a hydrolyzable group attached to the hydroxy group at carbon 25 of the molecule. The presence of a hydrolyzable group at

that position imparts on the resulting derivatives the desirable "slow-release" biological activity profile mentioned above. Other hydroxy functions occurring in the molecule (e.g. hydroxy functions at carbons 1 or 3) may be present as free hydroxy groups, or one or more of them may also be derivatived with a hydrolyzable group.

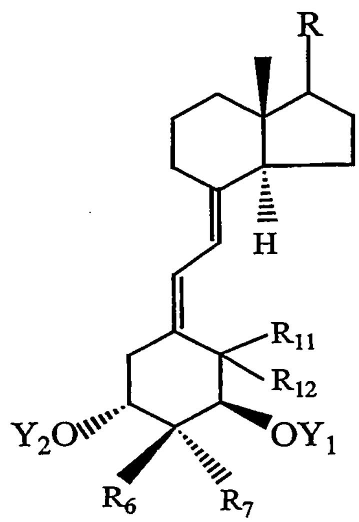
The "hydrolyzable group" present in the above-mentioned derivatives is preferably an acyl group, i.e. a group of the type Q^1CO-, where Q^1 represents hydrogen or a hydrocarbon radical of from 1 to 18 carbons that may be straight chain, cyclic, branched, saturated or unsaturated. Thus, for example, the hydrocarbon radical may be a straight chain or branched alkyl group, or a straight chain or branched alkenyl group with one or more double bonds, or it may be an optionally substituted cycloalkyl or cycloalkenyl group, or an aromatic group, such as substituted or unsubstituted phenyl, benzyl or naphthyl. Especially preferred acyl groups are alkanoyl or alkenoyl groups, of which some typical examples are formyl, acetyl, propanoyl, hexanoyl, isobutyryl, 2-butenoyl, palmitoyl or oleoyl. Another suitable type of hydrolyzable group is the hydrocarbyloxycarbonyl group, i.e. a group of the type Q^2-O-CO-, where Q^2 is a C_1 to C_{18} hydrocarbon radical as defined above. Exemplary of such hydrocarbon radicals are methyl, ethyl, propyl, and higher straight chain or branched alkyl and alkenyl radicals, as well as aromatic hydrocarbon radicals such as phenyl or benzoyl.

These modified vitamin D compounds are hydrolyzable in vivo to the active analog over a period of time following administration, and as a consequence regulate the in vivo availability of the active analog, thereby also modulating their activity profile in vivo. The term "activity profile" refers to the biological response over time of vitamin D compounds. Individual modified compounds, or mixtures of such compounds, can be administered to "fine tune" a desired time course of response.

As used herein the term "modified vitamin D compound" encompasses any vitamin D compound in which one or more of the hydroxy functions present in such a compound are modified by derivatization with a hydrolyzable group. A "hydrolyzable group" is a hydroxy-modifying group that can be hydrolyzed in vivo, so as to regenerate the free hydroxy functions.

In the context of this disclosure, the term hydrolyzable group preferably includes acyl and hydrocarbyloxycarbonyl groups, i.e. groups of the type Q^1CO- and Q^2-O-CO, respectively, where Q^1 and Q^2 have the meaning defining earlier.

Structurally, the modified vitamin D compounds encompassed may be represented by the formula **VII** shown below:



VII

where Y_1, Y_2, R_{11}, R_{12}, R_6 and R_7 are as previously defined herein with respect to formula **I** with the exception that R^5 in the side chain is $-OY_3$ and Y_3 is an acyl group or a hydrocarbyloxycarbonyl group, as previously defined herein.

Some specific examples of such modified vitamin D compounds include 2-substituted derivatives such as:

2-methylene-19-nor-1α,25(OH)$_2$-D$_3$-1,3,25-Triacetate where Y_1=Y_2=Y_3 and is CH_3CO; and R_6 and R_7 taken together is $=CH_2$; and R_{11} and R_{12} are both hydrogen;

2-methylene-19-nor-1α,25(OH)$_2$-D$_3$-1,3,25-Trihexanoate where Y_1=Y_2=Y_3 and is $CH_3(CH_2)_4CO$; and R_6 and R_7 taken together is $=CH_2$; and R_{11} and R_{12} are both hydrogen;

2-methylene-19-nor-1α,25(OH)$_2$-D$_3$-1,3,25-Trinonanoate where Y_1=Y_2=Y_3 and is $CH_3(CH_2)_7CO$; and R_6 and R_7 taken together is $=CH_2$; and R_{11} and R_{12} are both hydrogen;

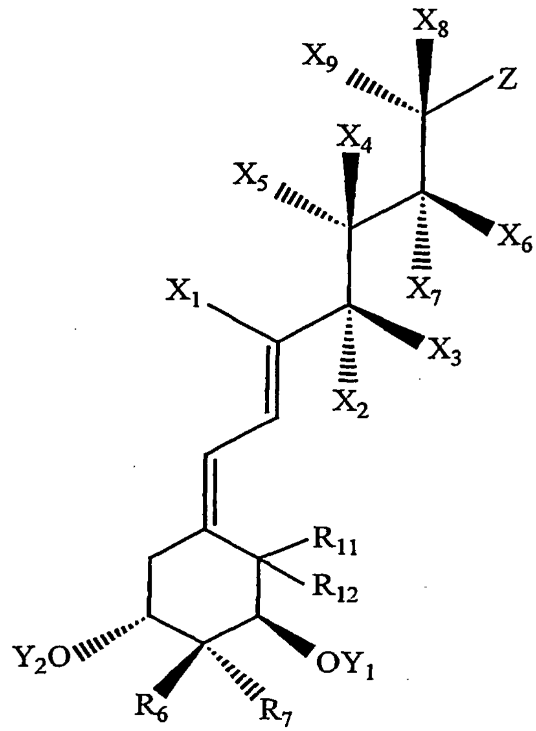
2-methylene-19-nor-1α,25(OH)$_2$-D$_3$-25-Acetate where Y_1=Y_2 and is H and Y_3 is CH_3CO, and R_6 and R_7 taken together is =CH_2; and R_{11} and R_{12} are both hydrogen.

These compounds can be prepared by known methods. See for example WO97/11053 published March 27, 1999.

Other 2-Substituted Compounds

In its broadest application, the present invention relates to any 2-substituted analogs of vitamin D which have the vitamin D nucleus. By vitamin D nucleus, it is meant a central part consisting of a substituted chain of five carbon atoms which correspond to positions 8, 14, 13, 17 and 20 of vitamin D, and at the ends of which are connected at position 20 a structural moiety representing any of the typical side chains known for vitamin D type compounds (such as R as previously defined herein), and at position 8 the 5,7-diene moiety connected to the A-ring of an active 1α-hydroxy vitamin D analog (as illustrated by formula **I** herein). Thus, various known modifications to the six-membered C-ring and the five-membered D-ring typically present in vitamin D, such as the lack of one or the other or both, are also embraced by the present invention.

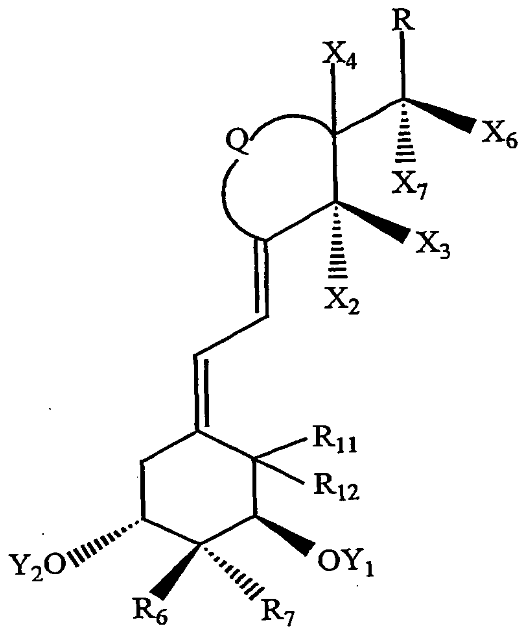
Accordingly, compounds of the following formulae **Ia**, are along with those of formula **I**, also encompassed by the present invention:



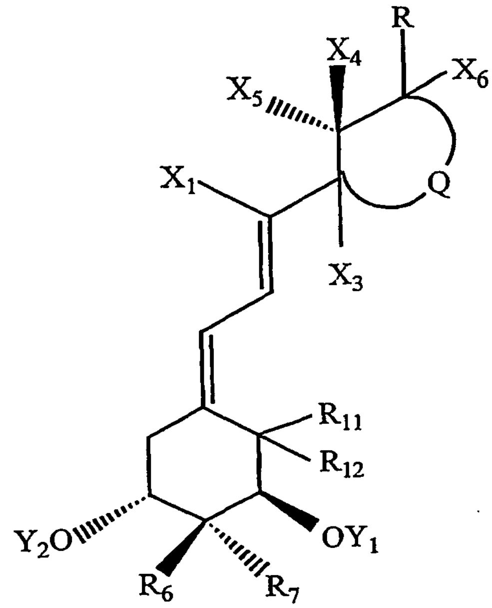
Ia

In the above formula **Ia**, the definitions of Y_1, Y_2, R_{11}, R_{12}, R_6, R_7 and Z are as previously set forth herein with respect to formula **I**. With respect to X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8 and X_9, these substituents may be the same or different and are selected from hydrogen or lower alkyl, i.e. a C_{1-5} alkyl such as methyl, ethyl or n-propyl. In addition, paired substituents X_1 and X_4 or X_5, X_2 or X_3 and X_6 or X_7, X_4 or X_5 and X_8 or X_9, when taken together with the three adjacent carbon atoms of the central part of the compound, which correspond to positions 8, 14, 13 or 14, 13, 17 or 13, 17, 20 respectively, can be the same or different and form a saturated or unsaturated, substituted or unsubstituted, carbocyclic 3, 4, 5, 6 or 7 membered ring.

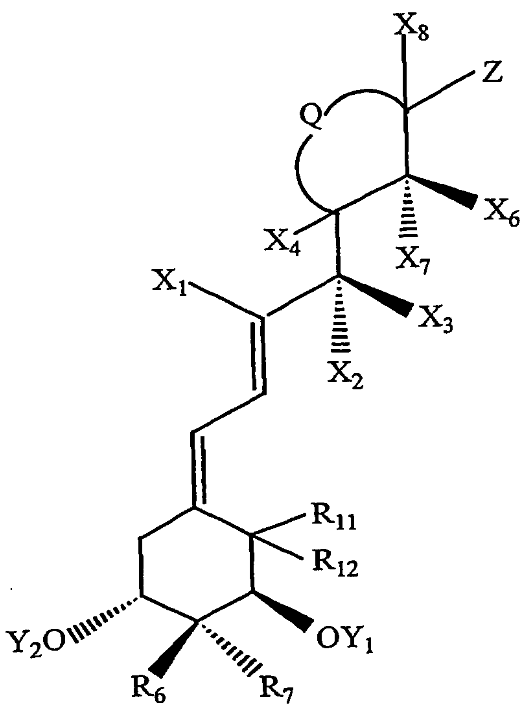
Preferred compounds of the present invention may be represented by one of the following formulae:


R
X4
X6
Q
X7
X3
X2
R11
R12
Y2O
OY1
R6
R7

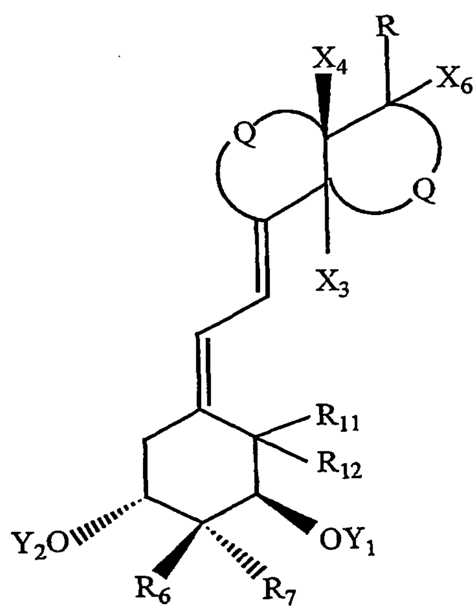
Ib


R
X4
X6
X5
X1
Q
X3
R11
R12
Y2O
OY1
R6
R7

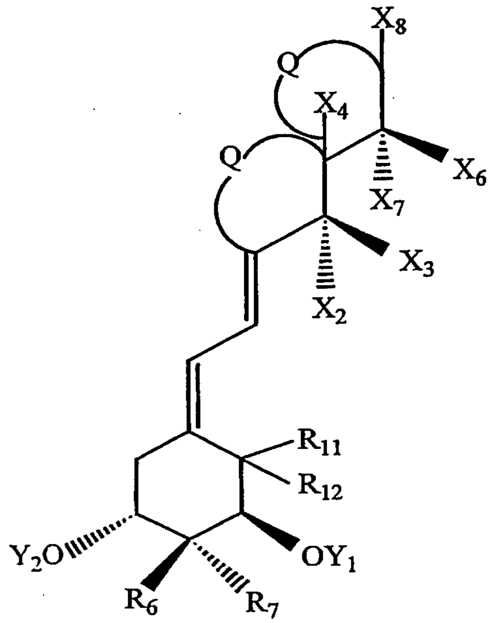
Ic


X8
Z
Q
X6
X4
X7
X1
X3
X2
R11
R12
Y2O
OY1
R6
R7

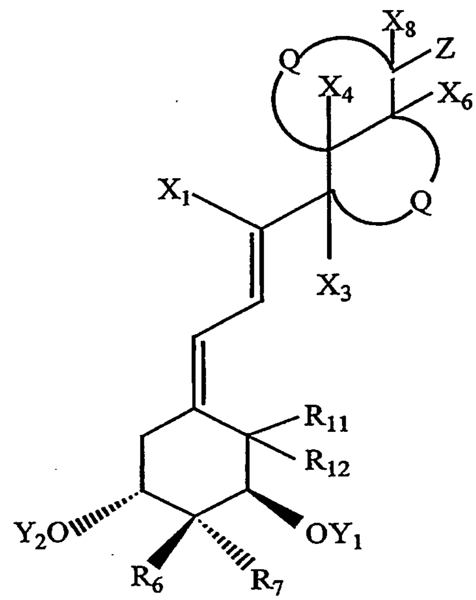
Id


R
X4
X6
Q
Q
X3
R11
R12
Y2O
OY1
R6
R7

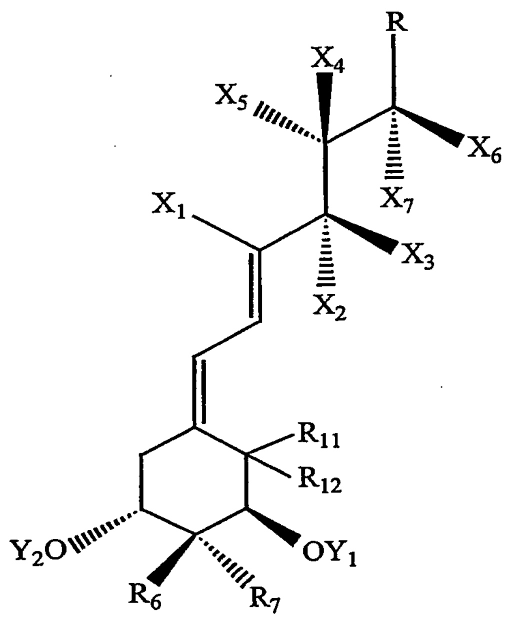
Ie



If



Ig



Ih

In the above formulae Ib, Ic, Id, Ie, If, Ig and Ih, the definitions of Y_1, Y_2, R_{11}, R_{12}, R_6, R_7, R. Z, X_1, X_2, X_3, X_4, X_5, X_6, X_7 and X_8 are as previously set forth herein.

The substituent Q represents a saturated or unsaturated, substituted or unsubstituted, hydrocarbon chain comprised of 0, 1, 2, 3 or 4 carbon atoms, but is preferably the group $-(CH_2)_k-$ where k is an integer equal to 2 or 3.

Methods for making compounds of formulae **Ia-Ih** are known. Specifically, reference is made to International Application Number PCT/EP94/02294 filed 7 July 1994 and published 19 January 1995 under International Publication Number WO95/01960.

For treatment purposes, the compounds defined by the formulae herein may be formulated for pharmaceutical applications as a solution in innocuous solvents, or as an emulsion, suspension or dispersion in suitable solvents or carriers, or as pills, tablets or capsules, together with solid carriers, according to conventional methods known in the art. Any such formulations may also contain other pharmaceutically-acceptable and non-toxic excipients such as stabilizers, anti-oxidants, binders, coloring agents or emulsifying or taste-modifying agents. The formulations may be administered orally, systemically or locally. If local, the compound may be administered in an immobilized form, as is well known in the art, or by Alzet mini-pump.

The compounds may be administered orally, topically, parenterally or transdermally. The compounds are advantageously administered by injection or by intravenous infusion or suitable sterile solutions, or in the form of liquid or solid doses via the alimentary canal, or in the form of creams, ointments, patches, or similar vehicles suitable for transdermal applications. Doses of from 0.01μg to 50μg per day of the compounds are appropriate for treatment purposes, such doses being adjusted according to the disease to be treated, its severity and the response of the subject as is well understood in the art. Since the new compounds exhibit specificity of action, each may be suitably administered alone, or together with graded doses of another active vitamin D compound -- e.g. 1α-hydroxyvitamin D_2

or D_3, or 1α,25-dihydroxyvitamin D_3 -- in situations where different degrees of bone mineral mobilization and calcium transport stimulation is found to be advantageous.

The compounds may be formulated as creams, lotions, ointments, topical patches, pills, capsules or tablets, or in liquid form as solutions, emulsions, dispersions, or suspensions in pharmaceutically innocuous and acceptable solvent or oils, and such preparations may contain in addition other pharmaceutically innocuous or beneficial components, such as stabilizers, antioxidants, emulsifiers, coloring agents, binders or taste-modifying agents.

The compounds are advantageously administered in amounts sufficient to effect the desired therapeutic result for a specified condition and route of administration, i.e. a "therapeutically effective amount." Dosages as described above are suitable, it being understood that the amounts given are to be adjusted in accordance with the severity of the disease, and the condition and response of the subject as is well understood in the art.

The formulations of the present invention comprise an active ingredient in association with a pharmaceutically acceptable carrier therefore and optionally other therapeutic ingredients. The carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient thereof.

Formulations of the present invention suitable for oral administration may be in the form of discrete units as capsules, sachets, tablets or lozenges, each containing a predetermined amount of the active ingredient; in the form of a powder or granules; in the form of a solution or a suspension in an aqueous liquid or non-aqueous liquid; or in the form of an oil-in-water emulsion or a water-in-oil emulsion.

Formulations for rectal administration may be in the form of a suppository incorporating the active ingredient and carrier such as cocoa butter, or in the form of an enema.

Formulations suitable for parenteral administration conveniently comprise a sterile oily or aqueous preparation of the active ingredient which is preferably isotonic with the blood of the recipient.

Formulations suitable for topical administration include liquid or semi-liquid preparations such as liniments, lotions, applicants, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes; or solutions or suspensions such as drops; or as sprays.

For asthma treatment, inhalation of powder, self-propelling or spray formulations, dispensed with a spray can, a nebulizer or an atomizer can be used. The formulations, when dispensed, preferably have a particle size in the range of 10 to 100μ.

The formulations may conveniently be presented in dosage unit form and may be prepared by any of the methods well known in the art of pharmacy. By the term "dosage unit" is meant a unitary, i.e. a single dose which is capable of being administered to a patient as a physically and chemically stable unit dose comprising either the active ingredient as such or a mixture of it with solid or liquid pharmaceutical diluents or carriers.

__Example__

Human bone samples discarded after surgical procedures from patients undergoing hip/knee replacement surgeries are obtained under an approved IRB protocol (Protocol #2001-055) and processed as described below. These bone pieces are otherwise routinely discarded as waste material during surgery.

The bone pieces are thoroughly washed and cleaned under sterile conditions using phosphate-buffered saline (PBS). These pieces are then cut to obtain smaller pieces (1-2 mm^3) and subjected to enzymatic digestion process to isolate osteoblasts as described below.

Osteoblastic cells are obtained from the bone pieces by collagenase digestion. Briefly, the bone samples are washed twice in PBS and dissected in about $1mm^3$ size fragments which are then sequentially digested in trypsin (1mg/mL) for ten minutes, followed by dispase (2mg/mL) for twenty minutes, and bacterial collagenase [Collagenase A] (3mg/mL) twice for thirty minutes in PBS at 37^0C in a water bath. Cells released by collagenase digestion are then washed, counted and grown to sub-confluence in $25cm^2$ cell culture flasks in 1:1 Ham's F12/Dulbecco's modification of Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. Cells are cultured at 37^0C in a humidified atmosphere of 5% CO_2/95% air. Medium changes are made every 2-3 days and cells are cultured until they are 80% confluent. Cells are then trypsinized, washed and frozen from early passages for evaluation using techniques as described below.

In order to evaluate the ability of osteoblasts to form bone nodules *in vitro*, cells are cultured in 6 well plates at 1- 3 $X10^5$ cells/well. Cells are cultured in the presence of either 1,25-$(OH)_2D_3$ or vitamin D analogs at various doses for 7 days. Complete medium changes are carried out twice during the 7-day period and the medium is supplemented with fresh compounds (either 1,25-$(OH)_2D_3$ or 2-methylene-19-nor-(20S)-1α,25-dihydroxyvitamin D_3 -- 2MD) during each medium change. At days 10 and 13, complete medium changes are carried out and the compounds are replaced by treatment with ascorbic acid (50 μg/ml) and β-glycerol phosphate (10 mM). A third dose of ascorbic acid and β-glycerol phosphate is added to the cultures on day 15 if needed. Following the culture period, cells are stained using the Von Kossa technique. Briefly, cells are stained with 5% silver

nitrate for 30 minutes in the dark, rinsed with distilled water, reduced with sodium carbonate/formaldehyde solution for 2 minutes and washed under tap water for 10 minutes. The cells are then stained with methyl green pyronin for 20 minutes, washed with water followed by two washes of absolute alcohol. By this method, bone nodule formation *in vitro* is confirmed by the presence of calcium phosphate (calcified matrix) that stains dark brown to black in the nodular regions of the culture(Marie[15,16], 1994; 1995; Shevde et al.[17], 2001). Bone nodule formation *in vitro* can be assessed quantitatively by various published procedures.

Results and Interpretation

Figure 1 illustrates photographs of the osteoblast cultures after 14 days of incubation and shows dramatically that very little change is introduced by 1,25-$(OH)_2D_3$ when provided at 10^{-8} molar. Thus, the native vitamin D hormone appears to have a minimum effect on the osteoblasts to form mineralized bone. Figure 2 provides a Von Kossa stained series of cultures with different concentrations of 1,25-$(OH)_2D_3$ or 2MD. These results clearly demonstrate that 2MD has a unique and strong action on stimulating the osteoblast cultures to form mineralized bone as revealed by the Von Kossa stain. Even at a concentration of 10^{-12} molar, 2MD produced a saturating degree of new bone formation. These results have been repeated with different human osteoblast cultures on several occasions with identical results and conclusions. Further, similar results have been obtained with primary mouse calvarial osteoblast cultures. It is evident that 2MD specifically and markedly induces osteoclastic-mediated bone formation. These results suggest that this compound and its related analogs can be used to stimulate osteoblast-mediated bone growth. This is confirmed by the ability of this compound to markedly stimulate bone mass accumulation in the ovariectomized animal (DeLuca U.S. Patent No. 6,306,844). The present results, however, demonstrate that this

accumulation of bone mass is due to a marked stimulation in the formation of new bone. It can be envisioned, therefore, that 2MD and its analogs might be extremely useful in stimulating callus formation and fracture healing. Thus, a patient who has fractured any portion of his/her skeleton could be treated orally, systemically, or directly with 2MD to facilitate fracture healing. One can envision providing 2MD in a slow-release form at the site of the fracture; thereby providing a slow-release form such as 2MD 25-acetate or in an osmotic minipump to deliver a small amount of this compound each hour, or could be implanted in an immobilized form or injected in an immobilized form into the fracture area. Further, these results suggest that this compound either provided systemically or placed at the site would markedly stimulate the growth and healing of bone transplants as for example in distraction osteogenesis procedures. One can also envision that this compound could be very useful in patients who have had implants or devises that are used to heal or hold bone in place.

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